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Title

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Abstract

Background

Bacterivores, mostly represented by protists and nematodes, are a key component of soil biodiversity involved in soil fertility and plant productivity. In the current context of global change and soil biodiversity erosion, it becomes urgent to suitably recognize and quantify their ecological importance in the maintenance of ecosystem functions.

Scope

Using meta-analysis tools, we aimed at providing a quantitative synthesis of the ecological importance of soil bacterivores on ecosystem functions. We also intended to produce an overview of the ecological factors that are expected to drive the magnitude of bacterivore effects on ecosystem functions.

Conclusions

Bacterivores in soil contributed significantly to numerous key ecosystem functions. We propose a new theoretical framework based on ecological stoichiometry stressing the role of C:N:P ratios in soil, microbial and plant biomass as important parameters driving bacterivore-effects on soil N and P availability for plants, immobilization of N and P in the bacterial biomass, and plant responses in nutrition and growth.

Keywords

Bacterivores; Protists, Nematodes; Microbial loop; Plant growth; Plant nutrition ; Ecological stoichiometry; Meta-analysis

1. Introduction

Soils are some of the most biologically diverse environments, encompassing about 25% of global biodiversity in terms of species (Coleman and Wall 2015; Decaëns 2010). One gram of soil can host 6000 different bacterial genomes, several hundred meters of fungal hyphae and a wide range of protists, nematodes, enchytraeids or mites (Jones et al. 2009; Lavelle and Spain 2001; Wall et al. 2010). Through their activities, soil organisms ensure key soil functions (Barrios 2007; Brussaard et al. 2007; Wagg et al. 2014) and contribute to many ecosystem services (de Vries et al. 2013; Kibblewhite et al. 2008).

Unfortunately, soils are also among the most threatened environments in terms of biodiversity loss (Kibblewhite 2012). Many pressures (soil erosion, land use change, overexploitation, pollution, biological invasion, etc.) have been identified as directly disturbing soil organism abundance, distribution and activity (Bossio et al. 2005; Dupouey et al. 2002; Foley et al. 2005; Gardi et al. 2013; Pimentel 2006; Schlaghamersky et al. 2014). A thorough understanding of the many roles of soil biodiversity is required in order to predict how these threats could damage ecosystem services accomplished by soil organisms.

A key component of soil biodiversity involved in soil fertility and plant productivity are bacterivores (Bardgett et al. 1999; Bonkowski 2004; Bonkowski et al. 2009; Brussaard 1997). These bacterivores are mostly represented by protists and nematodes (Ronn et al. 2012). Bacterivorous soil protists are generally unicellular heterotrophic eukaryotes, that were traditionally grouped into ciliates, flagellates, naked amoebae and testate amoebae (Darbyshire 1994). However, molecular studies reveal an enormous taxonomic diversity in soils (Geisen et al. 2014; Geisen et al. 2015), and only the ciliated protists are monophyletic, while flagellated, and naked, and testate amoeboid morphotypes repeatedly originated during protist evolution (Adl et al. 2005; Adl et al. 2012).

Despite their small size (average size 2-50 µm), protists can largely contribute to total soil faunal biomass (Bonkowski 2004). Nematodes are small multicellular eukaryotes (0.03-1 mm size range) and form a highly diverse monophyletic group including about one million of species (Lambshead 1993), making this phylum one of the most diverse animal taxa on Earth (Lambshead et al. 2004). Both bacterial-feeding protists and nematodes are widely distributed in terrestrial ecosystems and successful colonizers of soil niches (Cowling 1994; Geisen et al. 2014). It has been estimated than tens of millions of protists and hundreds of thousands of nematode individuals co-exist in only one forest square meter (Cowling 1994; Foissner 1999; Yeates 2007; Yeates 2003). Still, they remain poorly studied in comparison to other soil taxa (Coleman and Wall 2015; Couteaux and Darbyshire 1998). Only a fraction of protist and nematode species have been described yet, and their biology and ecology remain poorly known (Brussaard et al. 2007). It thus becomes urgent, in the current context of global change that is expected to alter soil bacterivore communities, to suitably recognize the species and to quantify their ecological importance in the maintenance of ecosystems.

Here, as a complement to detailed reviews published in the past (Bonkowski 2004; Bonkowski and Clarholm 2012; Bonkowski et al. 2009; Chen et al. 2007), we aimed at providing a quantitative synthesis of the ecological importance of bacterivores in soil on ecosystem functions using meta-analysis tools. We also intended to produce an overview of the ecological (biotic and abiotic) factors that are expected to drive the magnitude of their effects on ecosystem functions.

2. Meta-analysis - Data sources, extraction and analysis

We investigated the literature published in peer-reviewed journals before December 2014 through computer searches in available databases (Google Scholar and ISI Web of

Knowledge), with no restriction on the year of publication, that investigate bacterivore-induced effects. We used the following key-words (alone or in combination): soil, biodiversity, bacteria, bacterivore, bacterial-feeders/grazers, protozoa, protists, nematodes, predators, mycorrhiza, earthworms, nodules, nutrient, nitrogen, phosphorus, microbial loop. We also searched studies in the reference lists of relevant articles and reviews. We selected studies that (i) tested the effects of bacterivores on ecosystem functions by successfully manipulating soil bacterivores in controlled experimental conditions, (ii) measured at least one function of interest, and (iii) reported means of treatment and control. We excluded field studies that monitored soil bacterivore population changes after perturbation or during seasonal cycles, or eliminated soil bacterivores by adding biocides in the soil, freezing or sieving the soil without measuring the success of the elimination. We also excluded studies comparing low *versus* high soil bacterivore densities and modelling studies.

We analyzed the effects of bacterivores on 18 microbial and plant functions: soil microbial biomass (measured by fumigation or substrate-induced respiration), soil bacterial number (measured by plate counting), soil microbial basal respiration, microbial metabolic quotient (microbial respiration divided by microbial biomass carbon), soil phosphatase activity, soil net N mineralization, soil net P mineralization, plant growth (shoot and root biomass, shoot:root ratio) and plant nutrition (total N and P amounts expressed in mg-N or mg-P plant^{-1} and concentrations in shoot and root expressed in mg-N or mg-P g^{-1}). For each study, we recorded the mean values of these functions in presence/absence of bacterivores as well as experimental information (soil parameters, species, protocols, etc.). These data were extracted directly from tables, the text or figures using PlotDigitizer 2.6.4 software.

For each observation, we calculated the individual effect size (ES_i) from the natural log of the response ratio using Equation 1 (Hedges et al. 1999).

(1) $ES_i = Ln (T_i / C_i)$; where "T" was the treatment mean (presence of bacterivores) and "C" the control mean (absence of bacterivores) for the observation i.

This metric reflects a relative change in a function due to the presence of bacterivores. Positive and negative ES_i indicated respectively a positive and a negative effect of bacterivores on the function of interest. We then calculated the mean effect size (ES_m) using the equation 2 and estimated the 95% confidence interval around the ES_m using bootstrapping (999 iterations).

(2) $ES_m = (\Sigma ES_i) / n$; with "n" the total number of observations

ES_m was considered significant ($P < 0.05$) if its 95% confidence intervals did not overlap with zero. For a more intuitive understanding of the effects of bacterivores on functions, we also calculated the percentage of change from control (%CC) from the ES_m. We also tested the effect of the medium used by the experimenters in their studies. To do so, we split the database into 4 groups according to the medium: "agar" (or agarose), "humus", "sand" (mixed with organic matter or with nutrient solution) and "soil" (alone or amended with sand, organic matter and/or nutrient solution). We then calculated ES_m for each group and tested for significance among groups using the Kruskal-Wallis test at the $P<0.05$ level.

We also determined whether the presence of mycorrhizal fungi altered bacterivore-effects on plant nutrition and growth. We collected data from studies comparing the effects of bacterivores in the rhizosphere of plants infected or not by mycorrhizal fungi (7 studies, 14 observations). We did not include studies where the control (non mycorrhizal plant) was lacking. For each observation, we calculated the ES_i for mycorrhizal and non-mycorrhizal

plants and tested for significant difference in ES_m using the Kruskal-Wallis test at the $P<0.05$ level.

Linear meta-regressions were performed to tested the relationships between the bacterivore-induced effect size on total N (or P) amount in shoot (or root) (responses variables) and the bacterivore-induced effect sizes on shoot (or root) N (or P) concentration and shoot (root) biomass (explanatory variables). 95% confidence intervals were calculated for each regression. The normal distribution of residuals was tested using Shapiro test. Finally, we searched for publication bias using funnel plots. We tested the significance (P-value < 0.05) of regression between the bacterivore-induced ES *versus* the inverse sample size of the study for each function (Peters et al. 2006). A significant regression indicated that the funnel plot was asymmetric, corresponding to possible publication bias, which was the case only for respiration (P-value: 0.02). We also tested the relationships between experiment duration, which ranged from 3 to 561 days, and ES_m values using regression. Except for the concentration of N in roots, we did not find any effect of experiment duration on ES_m.

3. Quantitative synthesis of soil bacterial-bacterivore literature

We collected 41 experimental studies published since 1977 investigating the effects of soil bacterivores on ecosystem functions (Appendix 1). Among them, 38 and 17 studies investigated effects of bacterivores on N and P concentrations (either in soil or plant tissues), corresponding to ~93% and ~41% of reported studies, respectively (Figure 1). Around 66% of experimental studies co-inoculated bacteria and bacterivores in the rhizosphere of plants, using either herbaceous (73%) or woody (27%) species. According to our literature survey, in a period of 37 years, only 1.1 papers per year on average focused on the ecological functions accomplished by these organisms (without taking into account reviews and books). We

believe that the number of studies (and observations) is large enough to conduct a meta-analysis but remains critically limited regarding the significant roles of these organisms on ecosystem functioning (Bonkowski 2004; Chen et al. 2007).

This apparent lack of interest in soil bacterivores can be explained by the difficulties involved in observing, characterizing (extraction, enumeration and description) and manipulating these small-sized organisms in comparison to larger soil fauna (e.g. arthropods or earthworms) (Cowling 1994; Ekelund and Ronn 1994; Griffiths and Ritz 1988). This is particularly true for protists because the majority of taxa cannot be extracted from soils, and our taxonomic knowledge is mostly restricted to few cultivable species (Ekelund and Ronn 1994; Foissner 1999). Furthermore, this practical difficulty is enhanced by the heterogeneous and opaque nature of soils at the micro-spatial scale in contrast to aquatic environments. Soil protozoology and nematology thus require specific taxonomic skills and time-consuming microscopy in addition to expensive equipment (high-quality light-, or electron-microscopy). These methodological drawbacks together with the difficulties working under strictly sterile conditions have certainly constituted the main obstacles in the establishment of soil micro-food web experiments.

We also noted that contrasting experimental protocols have been used in these studies. For instance, experimenters used various types of medium (agar, sand, humus or soil) or experimental devices (Petri dishes, glass tubes, rhizoboxes or pots) for the growth of organisms (Table 1). The amount and composition (C:N:P ratio) of organic amendments and nutrient solutions were also highly different among studies as well as the choice of model species and the duration of the experiment (from 2 to 561 days). This high diversity in protocols made the identification of ecological factors that are likely to drive bacterivore-effects on ecosystem functions more difficult. On the other hand, it also allowed us to

194 estimate an overall bacterivore-effect representative of a wide range of environmental
195 conditions.
196
197 **4. Bacterivore-effects on the soil microbial community and nutrient availability**
198 *4.1. Microbial biomass*
199
200 Because protists and nematodes feed on bacteria, the presence of these organisms usually
201 induced a reduction in soil microbial biomass or bacterial abundance (Anderson et al. 1978;
202 Coleman et al. 1977; Darbyshire et al. 1994; Elliott et al. 1979; Zwart and Darbyshire 1992).
203 However, a number of studies showed that the presence of bacterivores can lead to higher
204 bacterial abundance (and biomass) due to a strong reduction of senescent cells (Elliott et al.
205 1980; Ingham et al. 1985; Sundin et al. 1990). The global effect of grazing on the soil
206 microbial biomass and bacterial abundance estimated by the meta-analysis was -16% and -
207 17% of control, respectively (Figure 2).
208 The effect of bacterivores on bacterial abundance has been shown to vary according to
209 bacterivore species (Ingham et al. 1985; Postma-Blaauw et al. 2005), soil texture (Elliott et al.
210 1980), incubation time (Elliott et al. 1980), supply and availability of labile carbon (Anderson
211 et al. 1978; Elliott et al. 1980) or nitrogen (Baath et al. 1981) and identity of bacterial taxa
212 (Glücksman et al. 2010; Griffiths et al. 1999; Ronn et al. 2002; Xiao et al. 2010), showing that
213 differences in bacterivore-effects on bacterial abundance are multifactorial. Certainly the long
214 co-evolution between bacteria and protists had a major influence on the evolution of prey-
215 capture mechanisms (Parry 2004) and bacterial defense strategies (Jousset 2011; Matz and
216 Kjelleberg 2005). In consequence, predicting bacterivore-effect on soil bacterial community
217 in a particular environment (or experimental set up) remains difficult.
218

219 *4.2. Microbial composition*

220

221 The whole composition of the soil microbial community drastically changed in the presence
222 of protists (Bonkowski et al. 2011; Ekelund et al. 2009; Griffiths et al. 1999; Koller et al.
223 2013c; Ronn et al. 2002; Rosenberg et al. 2009) and bacterial-feeding nematodes (Blanc et al.
224 2006; Djigal et al. 2010; Djigal et al. 2004; Postma-Blaauw et al. 2005). Unfortunately, most
225 published studies have a coarse taxonomic resolution limiting our ability to assess accurately
226 grazing effects on microbial composition. Usually, the presence of bacterivores changed the
227 relative abundance of dominant bacterial populations (Djigal et al. 2004), often increasing the
228 proportions of bacteria that are grazing protected, either by physical means, such as gram-
229 positive bacteria (Griffiths et al. 1999; Ronn et al. 2002; Weekers et al. 1993) and those that
230 make filaments such as actinomycetes (Rosenberg et al. 2009), or by chemical means (Jousset
231 et al. 2009; Jousset et al. 2010; Jousset et al. 2008; Mazzola et al. 2009). The ability of
232 bacterivores to alter the composition of the microbial biomass can feed back on microbial
233 function, such as nitrification (Djigal et al. 2010; Jousset et al. 2006) or phosphatase activity
234 (Djigal et al. 2004; Gould et al. 1979). For instance, using a DGGE technique based on PCR
235 amplification of the amoA gene, Xiao et al. (2010) found a significant shift in the community
236 composition of ammonia-oxidizing bacteria (AOB) from *Nitrosospira* sp. to *Nitrosomonas* sp.
237 in presence of nematodes bacterivores.
238 Selective grazing of cells has been proposed as the main mechanism causing a shift in
239 microbial composition in the presence of protists (Bonkowski et al. 2009). Bacteria evolved
240 different means to become grazing-resistant, which can be grouped in physical protections
241 such as bacterial shape and size (Bjornlund et al. 2012), cell wall resistance, and the ability of
242 cells to form micro-colonies, filaments or biofilms (Bonkowski et al. 2009; Jousset 2011); and
243 chemical protections, such as the production of pigments (Weekers et al. 1993) or of specific

toxins (Jousset et al. 2006; Jousset et al. 2009; Mazzola et al. 2009). In particular, the latter have been identified as main drivers of changes in bacterial community composition, since bacterivores have been shown to consume preferentially the competitors of the grazing-resistant taxa (Jousset et al. 2008). The differences in growth rates of bacterivorous protist (Ekelund 1996; Weekers et al. 1993) and nematode populations (Anderson and Coleman 1981; Blanc et al. 2006; Venette and Ferris 1998) according to bacterial species suggest that also the digestibility and nutritional values of preys constitute an important trait involved in bacteria-bacterivore interactions (Pussard et al. 1994).

On the other hand, bacterivores have developed different forms of feeding (direct interception, grasping, filter-, diffusion- or raptorial feeding); this appears as an important trait involved in selective grazing aptitude (Parry 2004; Ronn et al. 2012; Weisse 2002). While ciliates move out of toxic biofilms, amoebae graze directly within biofilms and grasping and raptorial feeders rather rely on bacteria they can dislocate from biofilm surfaces, while diffusion and filter feeders rely on the free-swimming bacteria (Ronn et al. 2012). In addition, certain bacterivore nematode taxa evolved a grinder in the terminal bulb which serves for crushing trapped bacteria (Fürst von Lieven 2003), thus providing a physical means to consume bacteria with thick cell walls that are largely undigestible for other bacterivores, like gram-positive bacteria.

Because bacterial-feeding nematodes can ingest around 20 cells during each suction of their continuously pumping pharynx (Ferris et al. (1997), it appears unlikely that nematodes actively select for specific prey taxa such as protists. Jousset et al. (2009), using mixed populations of mildly toxic wild-type and non-toxic gacS-deficient mutants of *Pseudomonas fluorescens* CHA0 in batch and rhizosphere systems, showed that the diet composition of the nematode *Caenorhabditis elegans* did not vary with changes in the frequency of the two bacterial strains, confirming its lack in selective compared to *Acanthamoeba castellanii* that

clearly preferred the non-toxic bacterial mutants. Accordingly, bacterial-feeding nematodes are more likely to alter bacterial community composition through passive mechanisms. The high variations in the head shape of Cephalobidae species recorded by De Ley (1992) supports this hypothesis. He investigated in detail how the shape of the labial probolae and the development of cephalic probolae at the nematode head allow passive food specialization, thereby optimizing niche partitioning among co-occurring bacterivore nematode species. Lastly, bacterivores have been suggested to modify the amount and composition of rhizodeposits (Sundin et al. 1990), which can in turn feed back on the composition of the bacterial community (Benizri et al. 2002; Puglisi et al. 2013).

4.3 Microbial activity and nutrient availability

The presence of bacterivores generally enhanced the overall microbial activity and turnover (Alphei et al. 1996; Coleman et al. 1978; Djigal et al. 2004; Kuikman et al. 1990). The meta-analysis revealed that the presence of bacterivores significantly increased soil microbial basal respiration and microbial turnover, measured as microbial metabolic quotient (respired-carbon relative to microbial biomass carbon) by +29% and 35% of control, respectively (Figure 2), indicating that the microbial carbon-use efficiency (CUE) strongly decreases in presence of bacterivores (Manzoni et al. 2012; Sinsabaugh et al. 2013). The main mechanisms are that (i) bacterivores can ingest senescent bacteria and contribute to maintain younger bacteria cells with higher metabolic activity (see discussion in Bonkowski (2004)) and (ii) they release undigested food particles, labile carbon and nutrients making the medium more favorable for bacterial re-growth (Griffiths 1994a; Pussard et al. 1994).

The effects of bacterivores on microbial communities have strong consequences for soil nutrient availability (Clarholm 1985a; Koller et al. 2013b; Kuikman and Vanveen 1989; Xiao

et al. 2010). We found that the presence of bacterivores almost doubled soil N (N_{min}) mineralization (x 1.8 in absence of plants) (Figure 2). Two main pathways of bacterivore-effects on soil nutrient availability do exist simultaneously: excretion of nutrients (direct way) according to the consumer-driven nutrient recycling (CNR) theory (Elser and Urabe 1999) and stimulation of the microbial activity and turnover as quoted earlier (indirect way). Using stable isotopes, Crotty et al. (2013) found that soil protists only have a production efficiency of 37%. Ferris (1997) found higher production efficiencies (ranging from 58% to 86%) for eight bacterivore nematodes species. Because bacterivores have to maintain stoichiometric homeostasis relative to their food, and loose carbon due to respiration, a great part of the ingested N will be ultimately released by bacterivores (Anderson et al. 1983; Darbyshire et al. 1994; Ferris et al. 1997; Ferris et al. 1998; Griffiths 1994b). Borkott (1989) found mass C:N ratios of 3.5:1, 4.7:1 and 5.6:1 for *Naegleria gruberi* (amoeba), *Colpoda steinii* (ciliate) and *Rhabditis* sp. (bacterivore nematode), respectively. Using recent values of global soil microbial stoichiometry (mass C:N ratios ranging from 5.5:1 to 7.4:1) (Cleveland and Liptzin 2007; Xu et al. 2013) and production efficiencies from Crotty et al. (2013) and Ferris et al. (1997), we calculated that theoretical ranges of N excretion would be 23-32%, 43-49% and 9-20% of assimilated-N for amoebae, ciliates and nematodes, respectively. The C:N ratios of both bacteria and bacterivores will thus directly influence the amount of assimilated-N excreted by the bacterivores (Elser and Urabe 1999).

Furthermore, the presence of bacterivores will increase microbial respiration and turnover (Coleman et al. 1978; Levrat et al. 1992), leading to lower microbial CUE and a decreased microbial C:N ratio. In addition, the C:N ratio of bacteria determines the respiration rates in presence of bacterivores, with C-losses increasing at decreasing C:N ratios (De Telegdy-Kovats 1932) leading to higher N release by bacterivores at narrow C:N ratios of the prey. However, the soil microbial biomass C:N:P has been shown to be relatively stable

compared to its resource (Cleveland and Liptzin 2007; Xu et al. 2013); and microbes keep strict homeostasis in respect to nutrient availability in soils (Griffiths et al. 2012). To maintain their stoichiometry, microbes can regulate their nitrogen-use efficiencies by releasing elements in excess depending on their CUE (Mooshammer et al. 2014a). The excess N may then become available for roots in presence of bacterivores (Kuzyakov and Xu 2013). However, small changes of microbial biomass stoichiometry can occur after shifts in the microbial community structure (Fanin et al. 2013). For instance, fast-growing bacteria (copiotrophs) have been suggested to exhibit lower biomass C:N:P ratios (higher nutrient requirements) than slow-growing ones (oligotrophs) (Elser et al. 2003; Hodge et al. 2000). Indeed, in line of the growth rate hypothesis (GRH) (Elser et al. 2003), N:P ratios in organism decrease with increasing growth rates due to elevated demands for P for the synthesis of P-rich ribosomes. Therefore, shifts in the dominance of fast- or slow-growing bacteria induced in presence of bacterivores can transfer to shifts in microbial biomass stoichiometry, with important consequences on the content of N and P available for plant growth.

Ferris et al. (1998) calculated that bacterial-feeding nematodes excreted NH_4-N with rates ranging between 0.0012 and 0.0058 µg-N ind.$^{-1}$ day^{-1} according to species, but in addition, considerable amounts of organic N (Norg) can be released by nematodes (Anderson et al. 1983). Wright (1975) found that that 3-14%, 15-34% and 35-45% of the total N (mean 13.7 µmoles N g^{-1} nematode fresh wt hour^{-1}) liberated in Ringer solution by the nematode *Panagrelus redivivus* were composed of urea, amino acids and proteins, respectively, while ammonium contributed 23-35% of total N-release. Large releases of organic N (in total and in proportion of total N) by nematodes may occur when nematodes exhibit high nitrogen-use efficiency (NUE) in order to balance their stoichiometry to bacterial biomass C:N:P ratios, explaining why Anderson et al. (1983) observed declines in excretion of Norg with decreasing resource availability (i.e. when nematode populations became older). Knowing that dissolved

344 Norg (especially low molecular weight N compounds) can be re-used by soil bacteria or taken
345 up by plants (Gallet-Budynek et al. 2009; Nasholm et al. 2009; Neff et al. 2003), bacterivore-
346 effects on soil N availability likely have been under-estimated because experimenters
347 generally focused only on NH_4.
348 Interestingly, we found a great variability in ES_i for N_{min} as shown by the 95% confidence
349 interval. In parallel, we found that when the substrate used in experiments was soil, the ES_m
350 was very high in comparison to humus, while in sand it exhibited intermediate values (Figure
351 3.A). Humus had the highest mass C:N ratios (mean ~39:1) while soils had the lowest ones
352 (mean ~15:1) and sand exhibited intermediate values (mean ~20:1). We therefore suggest that
353 bacterial growth in substrates with high C:N ratios (humus), and in absence of living roots,
354 will be mostly limited by N, leading to rapid immobilization of N released by bacterivores. In
355 contrast, in substrate with low C:N ratios (soil), bacterial growth will be limited by C and
356 bacterial N immobilization should decrease (Ferris et al. 1998).
357 We also found that protists exhibited significant higher effects than nematodes on N_{min} but the
358 highest values were found when both bacterivore groups were present in the microcosms
359 (Figure 4.A). Accordingly, the choice in bacterivore species appears as an important
360 experimental parameter explaining the magnitude of bacterivore-effects on N_{min}. The broad
361 classification of bacterivores is by far not accurate enough to predict how bacterivores impact
362 soil N cycling. For instance, Ferris et al. (1998) found high variation of different nematodes
363 species to net N mineralization. Predicting bacterivore-effects on nutrient availability
364 therefore remains difficult, especially when a mixture of bacterivore species is used.
365 Applying the same stoichiometric reasoning as for N (Borkott 1989; Cleveland and Liptzin
366 2007; Crotty et al. 2013; Ferris et al. 1997; Xu et al. 2013), theoretical ranges of excess-P
367 excretion would be 27-48%, 73-81% and 66-76% of assimilated-P for amoebae, ciliates and
368 nematodes, respectively. These estimates are higher than for N (except for nematodes) while,

in the meta-analysis, we found lower bacterivore-effects on P mineralization (P_{min}) (22% of control) in comparison to N (Figure 2). *Why did these theoretical differences in N and P excretion rates of bacterivores not match with N_{min} and P_{min} data provided by the meta-analysis?*

The theoretical liberations of N and P by bacterivores did not take into account biotic (microbial immobilization, microbial CUE, resource stoichiometry, etc.) and abiotic processes (adsorption of P, leaching of N, etc.) occurring after nutrient excretion by bacterivores, thus differentiating *gross* from *net* effects of bacterivores on nutrient availability. In humus (where the C:P ratio is high), the bacterivore-effect on P_{min} was negative, while in soil (where the C:P ratio is low) we found a positive effect of bacterivores on P_{min} (Figure 3.B). Hence, in a high C:P ratio environment, bacterial growth may be mostly limited by P and rapid immobilization of excreted P from bacterivores may have occurred. However, in contrast to N, P is poorly mobile in the soil solution and can be rapidly adsorbed by soil colloids (Hinsinger et al. 2011), reinforcing rapid immobilization of P in the microbial biomass and hampering the overall positive net effect of bacterivores on P_{min}.

5. Bacterivore-effects on plant performance

5.1. Plant growth and root architecture

Positive effect of bacterivores on plant growth have been observed for both herbaceous (Alphei et al. 1996; Djigal et al. 2004; Krome et al. 2009a) and woody (Bonkowski et al. 2001b; Irshad et al. 2011; Jentschke et al. 1995) plant species (see Table 1 for details). Our meta-analysis revealed a significant effect of bacterivores on shoot (+27% of control) and root biomass (+21% of control) without affecting the shoot:root ratio (Figure 2). Interestingly, the 95% confidence interval did not exhibit huge variation though many ecological factors have

been suggested in the literature to affect bacterivore-effects on plant growth. Both, protists and nematodes induced root growth of similar magnitude, but when both were present, their effects were significantly larger (Figure 4.C). We did not find such an additive effect for shoot biomass (Figure 4.B; *P*-value = 0.109).

Besides biomass, the presence of protists in the rhizosphere of plants has been shown to alter profoundly root architecture by promoting lateral root production (Jentschke et al. 1995; Kreuzer et al. 2006) (Table 1). Based on 11 studies, we found that bacterivores increased the number of root tips by 96% of control. However, this positive effect was greatly variable among studies (Table 1). Shifts in root architecture were also observed for bacterial-feeding nematodes but to a lesser extent than for protists (Cheng et al. 2011; Irshad et al. 2012; Irshad et al. 2011). For instance, while protists increased the number of root tips on average by a factor of 2.5, nematodes only increased the number of tips by a factor of 1.2 (Table 1). The presence of mycorrhizal fungi usually reduced the bacterivore effect on root length, specific root length, root area and number of root tips.

5.2. Plant nutrition

The positive effects of bacterivores on plant biomass could be mostly related to increased plant nutrient acquisition when protozoa and nematodes were inoculated in the soil (Bonkowski et al. 2009). Generally, we found substantial bacterivore-effects on the total amount of N in shoots and roots, *i.e.* +59% and +28% of control (Figure 2). Although the increase of plant N concentration in shoots by bacterivores was large (+25% of control) it was highly variable and not different from 0 (non-significant effect on this variable). By conducting linear meta-regressions, we found that ~34% ($P<0.001$) and ~65% ($P<0.001$) of the total variance of ES_m on total N amount in shoot (response variable) was explained by

bacterivore-effect on shoot biomass and shoot N concentration (explanatory variables), respectively, without significant interaction between the two explanatory variables (Figure 5.A). These results imply that in some experimental conditions, bacterivores enhanced the total shoot N amount by increasing the shoot N concentration while in others, bacterivores increased the shoot N amount by increasing shoot biomass, explaining why we found high variance in ES_m for shoot N concentration. Exceptions are the studies by Kuikman et al. (1991) and Alphei et al. (1996) where increased plant biomass production in presence of protists or nematodes was neither paralleled by an increased total uptake of plant N, nor by increased concentrations of N.

Interestingly, we found higher shoot N concentrations in experiments with bacterial-feeding nematodes than with protists (Figure 4.C). These results suggested that the increase in total shoot N amount was mostly caused by higher N concentrations in plant tissue when nematodes were inoculated. Together with the lower effects of nematodes on root architecture, the data suggest different mechanisms of plant responses to bacterivore nematodes or protists, and might explain why the combined effects of both bacterivore groups were generally larger than the effects of either group alone.

In respect to P, we found that bacterivores increased the total amount of P in shoots and roots by 38% and 55% of control, respectively (Figure 2). The data suggest differences in the internal plant cycling of newly-acquired N and P: the bacterivore-induced gain of N in plant tissues was mostly allocated to shoots (probably for the maintenance of high carbon fixation rates via photosynthesis) while the gain of P was more allocated to roots. Krome et al. (2009a) and Koller et al. (2013c) showed that the effects of protozoa on plant performance were more related to enhanced plant carbon fixation rather than nitrogen uptake. Compared to N, the effects of bacterivores on shoot P concentration (+30% of control) were less variable and different from 0, suggesting that surplus P made available by bacterivores was commonly

444 concentrated in plant tissues. Although the presence of bacterivores led to an important
445 increase in root P concentration (+23% of control), this effect was highly variable. The meta-
446 regression showed that ~16% (P<0.001) and ~75% (P<0.001) of the total variance of ES_m on
447 total P amount in roots (response variable) was explained by bacterivore-effects on root
448 biomass and root P concentration (explanatory variables), respectively without significant
449 interaction between these two explanatory variables (Figure 5.B). Some experimental
450 conditions, which remain to be known, may thus favor bacterivore-effects on nutrient
451 concentration in plant tissues (without affecting plant biomass) and others may promote
452 bacterivore-effects on plant biomass (and increase total N and P amount in plants).
453
454 *5.3. Plant reproduction & defense*
455
456 It is not surprising that positive effects of bacterivores on nutrient uptake and biomass of
457 plants will lead to increased plant reproduction. For instance, the presence of protozoa
458 increased the number of ears (+60%), seeds (24%) and the individual seed weight (+32%) of
459 barley plants, and the crop was even more tolerant to aphid herbivores (Bonkowski et al.
460 2001a). More recently, Krome et al. (2009a) showed that *A. castellanii* enhanced the seed
461 production of *Arabidopsis thaliana* (Brassicaceae) more than 3- and 7-fold compared to
462 treatments with bacteria and sterile grown plants, respectively. These results imply that gains
463 in plant fitness might be much more profound than increases of plant biomass and/or nutrient
464 contents in tissues indicate. Unfortunately, only these two papers investigated bacterivores
465 effects on plant reproduction. More studies are certainly needed to investigate bacterivore
466 effects on plant fitness and on interactions with herbivores and plant pathogens.
467
468 **6. Relationships between bacterivores and root-infecting symbionts**

469 *6.1. Bacterivores & Mycorrhizal fungi*

470

471 The effects of bacterivores on plant functions have been mostly investigated in the
472 rhizosphere of non-mycorrhizal plants (84% of reported studies) (Figure 1). This is a severe
473 shortcoming knowing that, in natural conditions, more than 80% of herbaceous and woody
474 plants are infected by soil fungi, mostly forming arbuscular mycorrhizal (Smith and Read
475 2008) and ectomycorrhizal symbioses (Marmeisse et al. 2004).
476 The formation of mycorrhizal roots is considered as the most widespread response of plants to
477 low soil nutrient availability (Plassard et al. 2011). By prospecting a large volume of soil
478 away from plant roots, and by releasing carboxylates (Finlay 2008) and enzymes (Courty et
479 al. 2006; Louche et al. 2010), mycorrhizal fungi have been often shown to significantly
480 enhance plant N and P acquisition (Plassard and Dell 2010; Tibbett and Sanders 2002;
481 Wallenda and Read 1999). The presence of mycorrhizal fungi can alter the positive effects of
482 bacterivores on plant performance through two main pathways: at a first glance, the presence
483 of mycorrhizal fungi is *a priori* likely to reduce the positive effect of bacterivores. The growth
484 of bacteria and bacterivores clearly depend on plant carbon allocation to roots. It has been
485 estimated that ca. 7-30% of net carbon fixation will be directly allocated to the root-infecting
486 fungal symbionts by the host plant (Jones et al. 2009; Leake et al. 2004). It is likely that the
487 amount of carbon released by root exudation will decrease in the presence of a mycorrhizal
488 partner (Meier et al. 2013; Olsson et al. 1996) with indirect detrimental effects on bacterivore
489 populations. Secondly, mycorrhizal fungi are known to profoundly change root biomass and
490 architecture (Brown et al. 2013). Usually, the presence of the mycorrhizal symbiont reduced
491 the stimulating effect of bacterivores on lateral root production (Table 1). Bacterivore effects
492 on plant nutrition through more complex root architecture can thus be altered by mycorrhizal
493 fungi.

494 However, this *a priori* hypothesis could not be confirmed by the meta-analysis. We did not
495 find any negative effect of mycorrhizal fungi on bacterivore effects on N and P concentrations
496 or total amount in plant tissues. In contrast, the ES_m of bacterivores on shoot and root biomass
497 were significantly reduced when the plants were in association with mycorrhizal fungi (Figure
498 6). This negative effect of mycorrhizal fungi on ES_m was very low for shoot biomass (-5%)
499 but substantial for root biomass (-104%), showing that even when mycorrhizal fungi reduced
500 the positive effect of bacterivores on root growth, the positive effects of bacterivores on plant
501 nutrition were still maintained. The few existing studies support this hypothesis for both
502 plants infected by arbuscular (Koller et al. 2013b; Koller et al. 2013c) and ectomycorrhizal
503 fungi (Bonkowski et al. 2001b; Irshad et al. 2012). These results also support the hypothesis
504 formulated by Bonkowski et al. (2001b) assuming that the mycorrhizal and the bacteria-
505 bacterivore mutualisms complement each other and plant resources are allocated to optimize
506 simultaneous exploitation of both mutualistic relationships. Because studies are lacking, it
507 was not possible to distinguish the effect of each mycorrhizal fungi type (arbuscular-, or ecto-
508 mycorrhiza) on bacterivore-effect sizes. Knowing that both fungal symbionts are
509 phylogenetically and physiologically distinct, this constitutes a serious shortcoming. More
510 studies are required, especially those focusing on different types of mycorrhizal fungi in
511 interaction with bacterivores.
512 It is well known that the ability of plants to take up organic N as source of N is greater in
513 presence of mycorrhizal fungi (Cappellazzo et al. 2008; Nasholm et al. 2009; Neff et al. 2003;
514 Plassard et al. 2000; Wallenda and Read 1999). Because significant amounts of dissolved
515 organic N (amino acids) are released by bacterial-feeding nematodes (Anderson et al. 1983;
516 Sundin et al. 1990; Wright 1975), we expect that the presence of mycorrhizal fungi will favor
517 the acquisition of some organic N- (and maybe P-) forms released by bacterivores, before
518 nutrients can be re-used by rhizosphere bacteria. Mycorrhizal plants could thus gain a

competitive advantage over microorganisms for soil N released by bacterivores. We did not find experimental study testing this hypothesis, but we believe that this organic N circuit should not be overlooked, and may constitute an important mechanism by which bacterivores improve mycorrhizal plant N nutrition.

6.2. Bacterivores & N_2-fixing plants

Because N usually limits plant productivity, biological N fixation is one of the most important functions of terrestrial ecosystems. Yet, little attention has been given to the potential role of bacterivores on symbiotic N fixation and legume growth (Appendix 1). Ramirez and Alexander (1980) showed that protists decreased the abundance of root-nodule bacteria in the rhizosphere of *Phaseolus vulgaris*. Lennox and Alexander (1981) found higher numbers of root nodules and growth of *P. vulgaris* when they used a biocide to suppress protists, but we can not judge the side-effects of the biocide, since no protist-specific biocides exist. High symbiotic N fixation efficiency usually requires high levels of available P because high rates of energy transfer take place in the symbiotic nodule (Drevon and Hartwig 1997; Vitousek et al. 2002). The meta-analysis showed that bacterial bacterivores increased soil P availability on average by 22% of control. We can hypothesize that protists and nematodes, by increasing soil P availability and subsequent plant P acquisition, might promote higher N fixation efficiency once the nodules are formed. The N:P ratio of nodules would then be an important proxy to predict how bacterivores in the vicinity of nodules may affect plant N uptake.

7. A 'stoichiometric' perspective on bacteria-bacterivore-plant interactions

7.1. Stoichiometric controls on bacterivore-effects on soil nutrient availability

Comparing the data of bacterivore effects on N- and P-availability point to an important role of stoichiometry if we want to predict how bacterivores impact ecosystem functions.

(1) Bacterivores have to maintain stoichiometric homeostasis, but especially protists have been shown to exhibit low production efficiency (Crotty et al. 2013). Accordingly bacterivores release a great part of ingested N and P in mineral form (Elser and Urabe 1999). The amounts of N- and P-release are expected to vary according to bacteria and bacterivores C:N:P ratios. For example, Cole et al. (1978) showed that amoebae were highly efficient at recycling microbially immobilized P to soil in comparison to bacterivore nematodes.

(2) Bacterivores often increase microbial turnover and further decrease microbial CUE. Microbes are strongly homeostatic in terms of their biomass C:N:P at the community scale (Cleveland and Liptzin 2007) and can regulate their nutrient-use efficiency by releasing elements in excess depending on their CUE (Mooshammer et al. 2014a) leading to a strict homeostasis with the available N- and P-levels in soil (Griffiths et al. 2012).

(3) Bacterivores can alter the soil microbial community structure. According to GRH (Elser et al. 2003), C:N:P ratios in organism vary with growth rates. Therefore, small changes in microbial stoichiometry can occur after shifts in the microbial community structure (Fanin et al. 2013) caused by bacterivores.

(4) Soils with high C:N or C:P ratios may be nutrient deficient for bacterial growth, favoring rapid immobilization of newly-mineralized N or P from bacterivores (Ferris et al. 1998). Soils with low C:N or C:P ratios may induce energetic starvation for bacterial growth, reducing bacterial N and P immobilization. The net effects of bacterivores on soil N and P mineralization therefore depend on energy available in terms of labile plant C, either provided by plant roots, or derived during initial phases of litter decomposition (Bonkowski and Clarholm 2012).

7.2. Stoichiometric controls on bacterivore-effects on plant nutrition and growth

Clarholm (1985b) and Bonkowski (2004) proposed theoretical frameworks describing how bacterivores impact plant functions. The 'soil microbial loop' hypothesis (Clarholm 1985a) described nutritional mechanisms, quoted earlier, by which bacterivores may increase plant nutrition and growth. Because bacterivores can enhance root growth without increasing plant nutrient concentrations (Alphei et al. 1996; Jentschke et al. 1995; Kuikman et al. 1991), Bonkowski (2004) proposed a 'hormonal' hypothesis based on non-nutritional mechanisms where protists increase lateral root branching by altering microbe-root communication, with strong consequences on plant internal auxin metabolism (Krome et al. 2009b). Both nutritional and non-nutritional mechanisms will occur concurrently in the rhizosphere of plants and are not exclusive of each other. However, it is unlikely that they contribute to the same magnitude to bacterivore-effects under different experimental conditions. For instance, we showed that the increase in total N amount in shoots induced by bacterivores was either explained by higher shoot N concentration or higher shoot biomass. We found similar partitioning in explanatory variables for bacterivore-effects on total P amount in roots. We believed that ecological stoichiometry can help to understand these patterns.

Ecological stoichiometry describes the importance of the relative proportions between elements for the growth of organisms (Elser et al. 2000; Gusewell 2004; Sardans et al. 2012; Zechmeister-Boltenstern et al. 2015). Plant C:nutrient ratios are assumed to decrease with increasing plant relative growth rate (Agren 2004; Elser et al. 2000). This was conceptually presented by Ågren (2008) as a linear relationship between these two plant traits (Figure 7.A). From this graph, we defined the "critical ratio" (R_{cri}) which designates the C:nutrient ratio above which plant growth is null (nutrient limitation).

For a certain plant species, the major cause of natural high C:nutrient ratios in plant tissues is assumed to be nutrient availability in soil (Ågren 2008). Nutritional mechanisms by bacterivore activity rely on a relatively high release of nutrients from consumed microbial biomass. Such conditions are experimentally met when detritus of narrow C:nutrient ratio is added to soil, supporting high levels of microbial growth and a subsequent substantial release of nutrients by bacterivores (Bonkowski et al. 2000). When the readily available organic C from the amendments is depleted, microbial biomass turnover will release nutrients for plant uptake, and nutrient release will be further enhanced in presence of bacterivores.

Under more natural soil conditions the nutritional mechanisms should prevail when nutrients are poorly available for plants, *i.e.* when competition for N and P between microbes and plants is strong. For example, Koller et al. (2013a) added litter of high C:N ratio (*i.e.* low quality, LQ) or low C:N ratio (high quality, HQ) to soil inoculated with bacteria or with bacteria and the amoeba *A. castellanii,* and planted with *Plantago lanceolata*. By ^{13}C-labeling of the plants, they showed that plants in presence of high C:N ratio substrates allocated 12% more recently fixed photosynthates to roots in presence of protists compared to controls. Subsequently, $^{13}CO_2$ respiration losses from the soil more than doubled, because the excess release of root C could not be fixed in microbial biomass due to nutrient deficiency. Here the presence of bacterivores caused a 17% decrease in plant C:N ratio which was likely due to two parallel processes, an increased nitrogen uptake and an excess energy expenditure towards rhizosphere C-allocation.

The non-nutritional mechanisms suggest that bacterivores stimulate higher root production and relatively more C-fixation before or without an increase in plant nutrient concentration. The formation of lateral roots and the resulting root branching requires substantial plant investment of photosynthates and nutrients: N for proteins, P for ribosomes and energy synthesis. Manipulating nutrient availability to plants through the addition of HQ litter to soil,

Koller et al. (2013a) found a substantially higher increase of root biomass (x1.8) (and increased root surface area) compared to shoot biomass (x1.3) in treatments with bacterivores compared to control. In contrast to the LQ treatment, the plant C:N ratio increased by 14%, *i.e.* plant biomass increased while the nutrient concentration decreased. This relatively wider plant C:N ratio might have been a result of a more efficient photosynthesis. At the same time, due to greater nutrient availability in low C:N substrates, microbial biomass in the plant rhizosphere incorporated substantially more ^{13}C from recent photosynthates. This may be observed when nutrients are highly available for plants, *i.e.* when competition for N and P between microbes and plants is low.

The relative dominance of nutritional and non-nutritional mechanisms induced by presence of bacterivores can be plotted along a soil C:N and C:P co-gradient likely to drive (i) plant-microbe competition towards N and P and (ii) plant stoichiometry status at the beginning or during co-inoculation experiments (Figure 7.B). The term *dominance* refers to the relative importance of mechanisms to initiate and drive bacterivore-effects on plants. We distinguished four contrasting cases. In case 1, plants exhibit C:N and C:P ratios above their respective critical ratios, and are strongly limited by N and P (the relative growth rate is therefore null). This case may happen when plants grow in soils with high C:N and C:P ratios. Under such soil conditions, microbes are strongly limited by N and P and expected to exhibit high NUE and PUE (Mooshammer et al. 2014b), and consequently the competition for N and P will be shifted in favour of microbes (Kuzyakov and Xu 2013). The experiment of Koller et al. (2013a) cited above, demonstrates that plants under nutrient limitation will allocate relatively more photosynthates belowground, thus providing energy for potential root symbionts or priming effects on soil organic matter. Under these circumstances the consumption of microbes by bacterivores will lead to increased microbial turnover and a decreased microbial CUE. Plants will primarily benefit from the released N and P by

643 bacterivores. In cases 2a and 2b, plants have C:N or C:P ratios just below their respective
644 critical ratios; they are thus strongly limited by N or P according to soil C:N:P ratios. Similar
645 mechanisms as described above may occur for other plant limiting nutrient concentrations,
646 but according to plant species stoichiometric behavior, an excess uptake of the non-limiting
647 nutrient may also occur (Ågren 2008). In cases 3a and 3b, plants grow in soils with
648 intermediate C:N or C:P ratios, respectively. Microbes become limited by carbon and their
649 NUE and PUE may decrease, while plants are limited by N or P but their C:nutrient ratios are
650 below the critical ratios. The presence of bacterivores, and the subsequent changes in nutrient
651 availability and root architecture, may result in both nutrient storage and biomass production,
652 according to species stoichiometric behavior (co-dominance of mechanisms). In case 4,
653 microbes are strongly limited by carbon (NUE and PUE are expected to be low) and plants
654 exhibit low C:N and C:P ratios. This may happen in substrates with low C:N and C:P ratios
655 (or soils with low availability of labile C). Plant nutrient concentrations are high enough to
656 cause an increase of plant biomass (dilution of plant nutrient concentration can occur), but
657 also lateral root production (*i.e.* root surface area) for more efficient nutrient uptake in
658 response to bacterivores (dominance of non-nutritional mechanisms). This case is expected to
659 happen when plants grow in a relatively nutrient-rich environment (Jentschke et al. 1995), or
660 when plants experience nutrient imbalances which is critical, especially at early life stages
661 (Fenner and Lee 1989; Hanley and Fenner 1997).
662
663 **8. Conclusion & Perspectives**
664
665 In this meta-analysis, we showed that bacterivores in soil contributed significantly to
666 numerous key ecosystem functions. We suggest a theoretical framework based on ecological
667 stoichiometry stressing the role of C:N:P ratios in soil, microbial and plant biomass as

668 important parameters driving bacterivore-effects on soil N and P availability for plants,
669 immobilization of N and P in the bacterial biomass, and plant responses in nutrition and
670 growth. Unfortunately, it has not yet been possible to test this 'stoichiometric' hypothesis
671 using meta-analysis tools because of insufficient data on soil and plant C:N:P ratios. More co-
672 inoculation experiments focusing on bacterivore-effects on plant nutrient acquisition in
673 various environmental conditions are therefore needed. In addition, we propose three outlooks
674 to contribute to our understanding of bacterivore functions in terrestrial ecosystems.
675 *From population to community-level experiments* – Studies investigating the effects of
676 bacterivores on ecosystem functions usually used one model species. In this meta-analysis, we
677 found that 57% of studies focusing on protists effects selected the *A. castellanii* species as a
678 model, corresponding to 28% of all studies reported in the meta-analysis. This species was
679 frequently used because it has a ubiquitous worldwide distribution (Geisen et al. 2014) and
680 has strong effects on the activity and composition of the soil microbial community (Griffiths
681 et al. 1999; Kreuzer et al. 2006; Rosenberg et al. 2009) and on root architecture (Bonkowski
682 and Clarholm 2012). Concerning bacterial-feeding nematodes, the genera *Mesodiplogaster*,
683 *Rhabditis*, *Acrobeloides* (and several other *Cephalobidae* genera), were frequently used. On
684 average, we found that 38% of studies used a mixture of different protists or nematodes
685 species. Only 6 studies (15%) investigated soil protists and nematodes together and only 2 of
686 them did it in the rhizosphere of a plant (Figure 1).
687 It is recognized that the effects of bacterivores on microbial community composition (Djigal
688 et al. 2004; Ronn et al. 2002), soil N mineralization (Ferris et al. 1998) or plant performance
689 (Bonkowski and Clarholm 2012; Cheng et al. 2011; Irshad et al. 2011) are species-specific.
690 Also, biological interactions among bacterivores are diverse and often antagonistic (Ronn et
691 al. 2012). Recent studies indicate that intraguild-predation between 'bacterivores', especially
692 antagonistic interactions between protists and nematodes are quite common and not

693 unidirectional. Bacterivore nematodes are known to prey on protists (Anderson et al. 1978;
694 Bonkowski et al. 2000; Neidig et al. 2010; Ronn et al. 2012) and vice-versa (Bjornlund and
695 Ronn 2008; Neidig et al. 2010). The population density of one bacterivore taxon can thus
696 increase on the cost of other another bacterivore (Anderson and Coleman 1981).
697 Unfortunately, the effect of within-trophic group diversity of the soil bacterivore community
698 on ecosystem function has rarely been investigated (Postma-Blaauw et al. 2005; Saleem et al.
699 2012). As a consequence, we are still far from predicting which bacterivore community
700 attribute(s) drive(s) the overall effect of the bacterivore community on ecosystem functions. A
701 number of community attributes might potentially drive the bacterivore community effects,
702 such as (i) species richness, (ii) keystone groups with specific ecology (*e.g.* amoebae), (iii)
703 keystone species (*e.g. Acanthamoeba castellanii*), (iv) particular trait dissimilarity (head
704 morphology, feeding types, body size, demographic parameters; etc.) or (v) multi-trait
705 dissimilarity (Rao's dissimilarity coefficient). Identifying the most relevant attributes of soil
706 bacterivore communities driving ecosystem functions should help us to get closer to
707 predicting the actual effects of bacterial bacterivores in the fields.
708 *Kinetic experiments to monitor bacterivore-induced effects on plant performance* – Most
709 studies investigated the effect of bacterivores on plant performance after a specific period of
710 growth. Kinetic experiments are scarce because monitoring bacterivore-effects on plant
711 growth or nutrition requires a high number of replicates, which is difficult from a technical
712 point of view. However, this type of studies might provide crucial information on the
713 sequence of plant and microbial responses in the presence of bacterivores, and the dynamic
714 and persistence of bacterivore-effects on ecosystem functions (Krome et al. 2009a). For
715 instance, Ingham et al. (1985) observed a temporary effect of nematodes (*Pelodera* sp. and
716 *Acrobeloides* sp.) on plant (*Bouteloua gracilis*) growth. Kinetic experiments conducted under
717 contrasting soil N and P availability would be optimal to test the *stoichiometric* hypothesis.

Emphasize on P and ecological factors – P is essential for plant growth and may be limiting in many environments. In contrast to N, P is characterized by its relative immobility in soil and the very low concentration of its available form in the soil solution (Hinsinger 2001; Hinsinger et al. 2011). Here, we found that N was 2.3-times more studied than P in both soil and plant tissues. This is a serious deficiency given that plant P acquisition efficiency is often based on biological interactions between plant roots and rhizosphere organisms. Using P radio-isotopes is probably the best way to better understand how bacterivores affect P cycling at the soil-plant interface. Also, the literature indicates that a large number of other ecological factors are likely to impact bacterivore-effects on ecosystem functions: bacterivore species (Cheng et al. 2011; Ferris et al. 1997), soil properties (Ekelund and Ronn 1994), plant species, or cultivars (Somasundaram et al. 2008), the presence of symbiotic mutualists (Herdler et al. 2008; Irshad et al. 2012) or other free fauna (Bonkowski and Schaefer 1997; Tao et al. 2009), time after inoculation (Krome et al. 2009a), etc. Unfortunately, the number of observations for each factor was too low or difficult to aggregate to perform a meta-analysis. For instance, it was impossible to test the effect of initial bacterivore biomass (or density), soil properties (pH, texture, carbon content, water content; etc.), plant species or cultivar, or the presence of rhizosphere organisms (*e.g.* mycorrhizal fungi or rhizobia) on bacterivore-induced effects on ecosystem functions. Conducting more detailed studies on bacteria/root/bacterivore interactions should deeply improve our understanding of soil food web roles in ecosystems functions, and ultimately help us to predict how the erosion of soil biodiversity will affect ecosystem services in the future.

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Table 1. Effects of bacterivores on plant root parameters (% of control).

References	Years	Plant species	Bacterivores	Modalities within studies	Root architecture (min − max)*			
					Length (m)	SRL (m g^{-1})	Area (m^2)	Tips (number)
Jentschke et al.	1995	*Picea abies*	Protist	- *Lactarius rufus*	135 − 180	38 − 70		130 − 220
				+ *Lactarius rufus*	22 − 45	2 − 36		30 − 45
Bonkowski et al.	2001	*Picea abies*	Protist	- *Paxillus involutus*	62	56		50
				+ *Paxillus involutus*	32	-32		32
Bonkowski & Brandt	2002	*Lepidium sativum*	Protist	*Main roots*	15			
				Lateral roots	421			286$
Kreuzer et al.	2006	*Oryza sativa*	Protist	*Total roots*	17			-37$
				Root diameter > 4mm	-43			-56$
				Root diameter < 4mm	195			1140$
Herdler et al.	2008	*Oryza sativa*	Protist	- *Glomus intraradices*		-27		
				+ *Glomus intraradices*		-15		
Krome et al.	2009	*Lepidum sativum*	Protist	*Agar medium*				100
				Soil medium	28 − 62			88 − 91
Irshad et al.	2011	*Pinus pinaster*	Nematode	-			-18	3
Cheng et al.	2011	*Oryza sativa*	Nematode	*Day 14*	14 – 22			26 – 43
				Day 20	2 – 15			-3 – 12
Irshad et al.	2012	*Pinus pinaster*	Nematode	- *Hebeloma cylindrosporum*	37 – 41		20 − 35	31 − 46
				+ *Hebeloma cylindrosporum*	20 − 32		16 − 20	18 − 36
Koller et al.	2013b	*Plantago lanceolata*	Protist				31	
Ranoarisoa et al.	2015	*Pinus pinaster*	Nematode	- *Hebeloma cylindrosporum*	7 − 32		9 − 22	1 − 15
				+ *Hebeloma cylindrosporum*	6 − 31		2 − 23	4 − 23
Mean					**56**	**16**	**16**	**96**

*Specific root length (SRL)

$Number of tips estimated from number of roots

Figure 1. Number of studies included in the meta-analysis (total and per criteria).

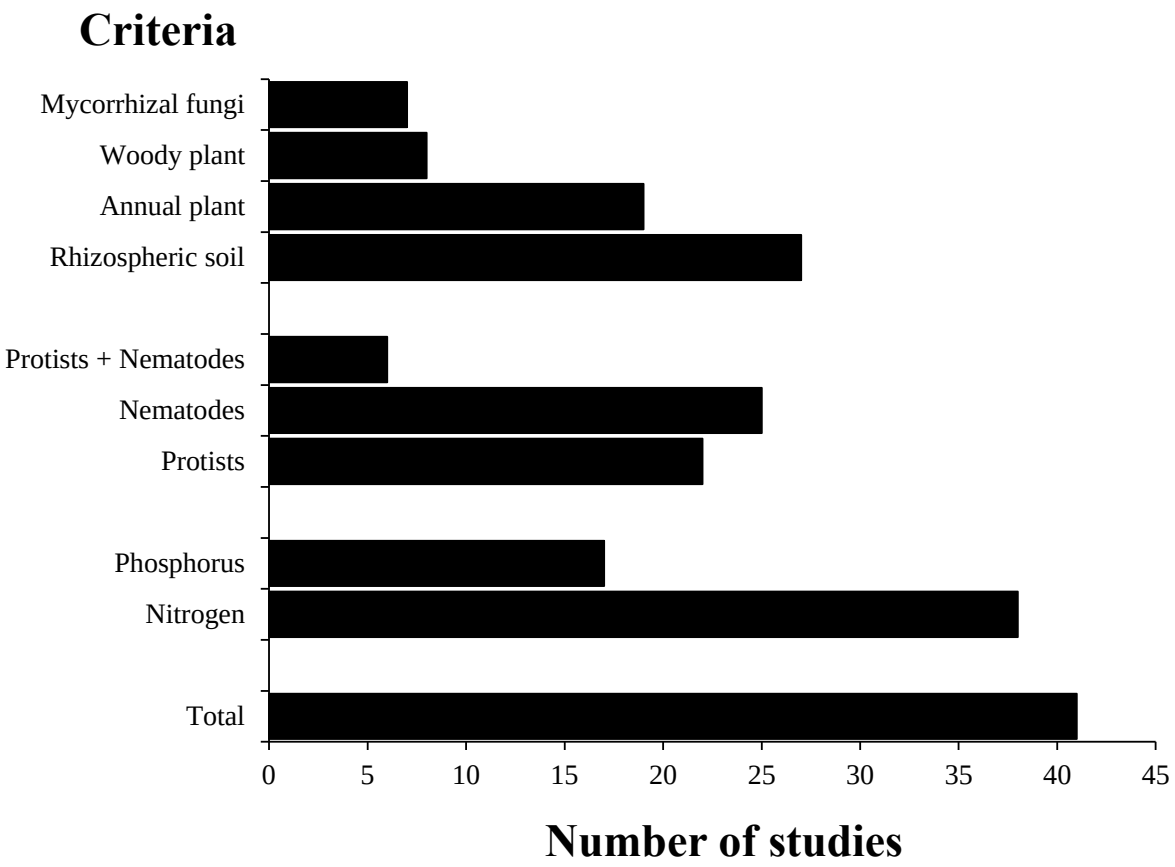


Figure 2. Mean effect sizes (ES_m) of bacterivores on ecosystem functions. White and black circles indicate significant (different from zero) and non-significant ES_m, respectively. Horizontal bars correspond to 95% confidence intervals. Bold numbers above circles specify the ES_m expressed as % of control (%CC). Italic numbers between brackets specify the total number of observations for each function. M_{mic} : microbial biomass; B_{num} : bacterial number; Q_{mic} : microbial metabolic quotient; N_{min} : net N mineralization; P_{min} : net P mineralization; S:R : shoot:root ratio.

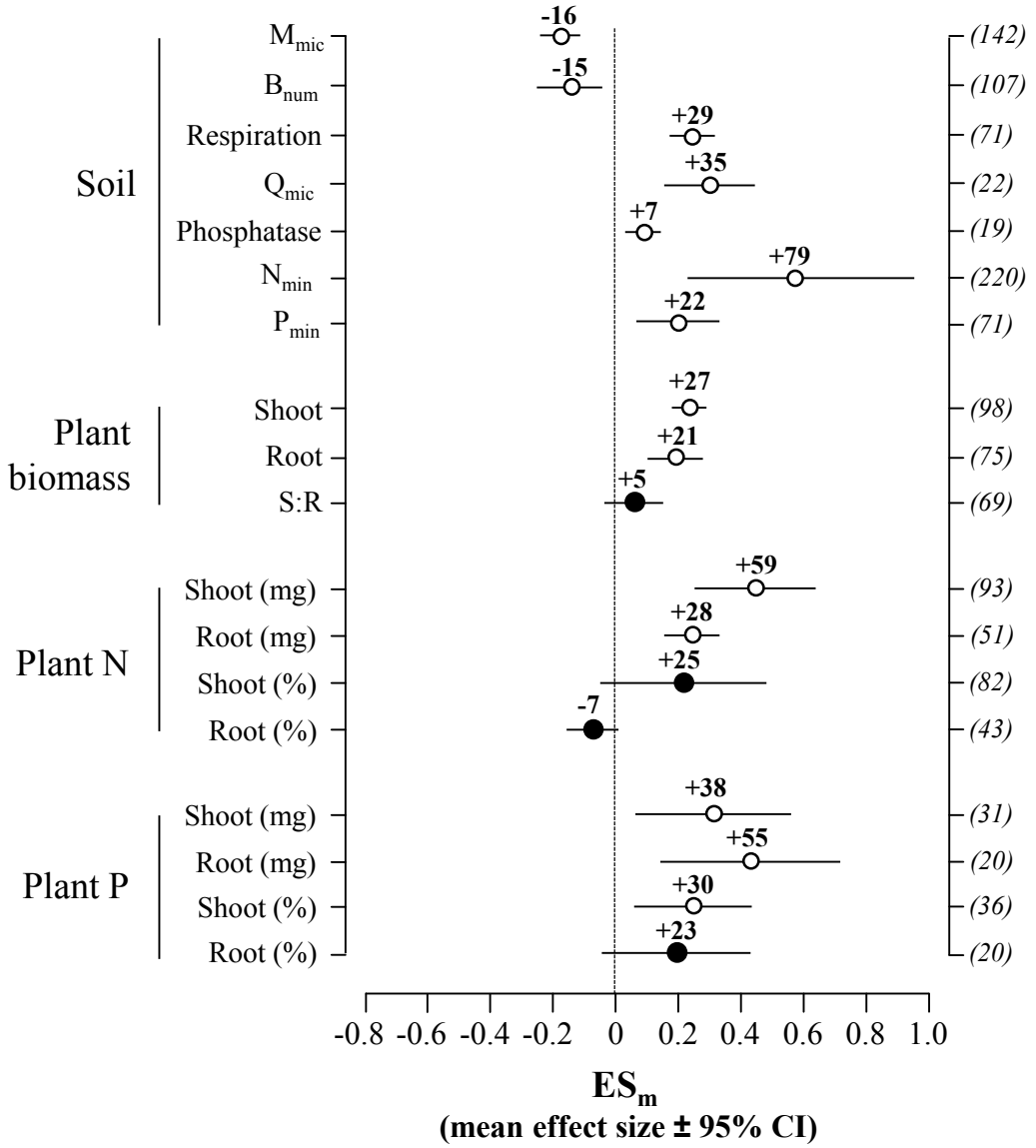


Figure 3. Changes in bacterivore-induced effect size (mean) on soil net N (A) and P (B) mineralization according to media types used in experimental studies. Letters (a and b) indicate significant differences in ES_m between media types according to a Kruskal-Wallis test at $P<0.05$.

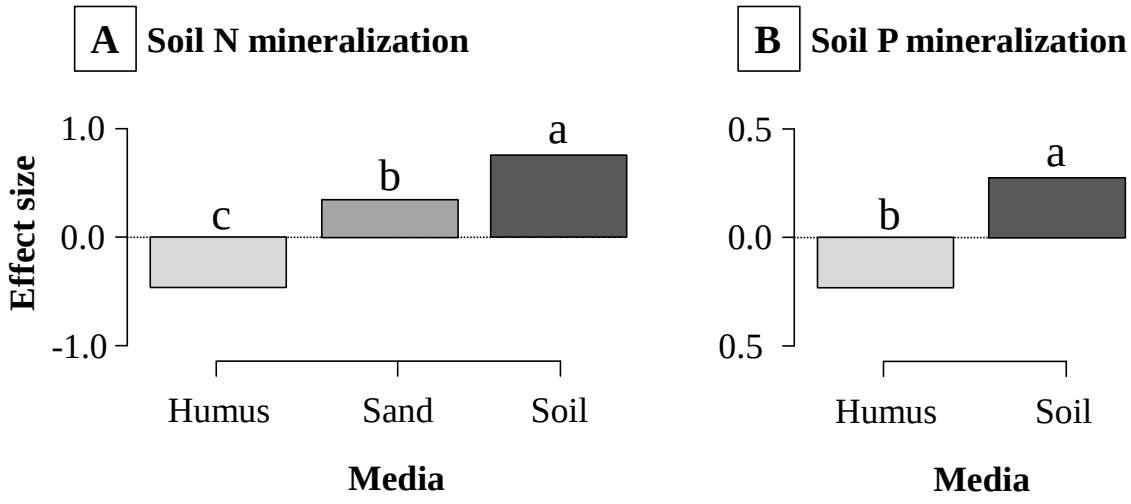


Figure 4. Changes in bacterivore-induced effect size (mean) on shoot N mineralization (A), root biomass (B) and shoot N concentration (C) according to the presence of bacterial-feeding protists (Pr), nematodes (Nem) alone or together (Pr + Nem). Letters (a and b) indicate significant difference in ES_m among media types according to a Kruskal-Wallis test at $P<0.05$.

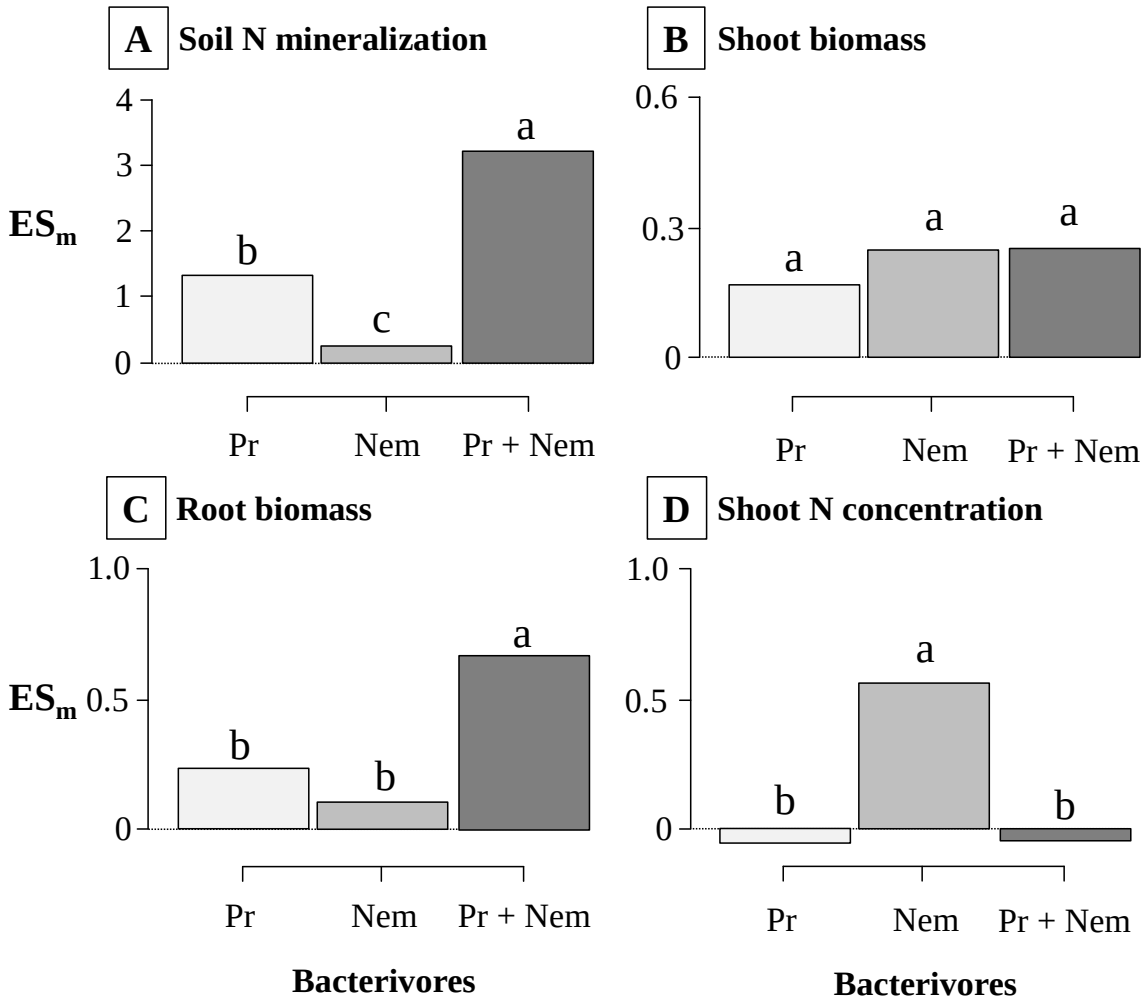


Figure 5. Multiple linear regressions performed on individual effect-size (ES_i). (A) ES_i of total N amount in shoot (response variable) as a function of ES_i of shoot biomass and ES_i of shoot N concentration (explanatory variables). (B) ES_i on total P amount in root (response variable) as a function of ES_i on root biomass and ES_i on root P concentration (explanatory variables). $\%_{expl.}$ indicates the percentage of total variance of the response variable explained by each explanatory variable and by their interaction. ***P<0.001 (n= number of observations).

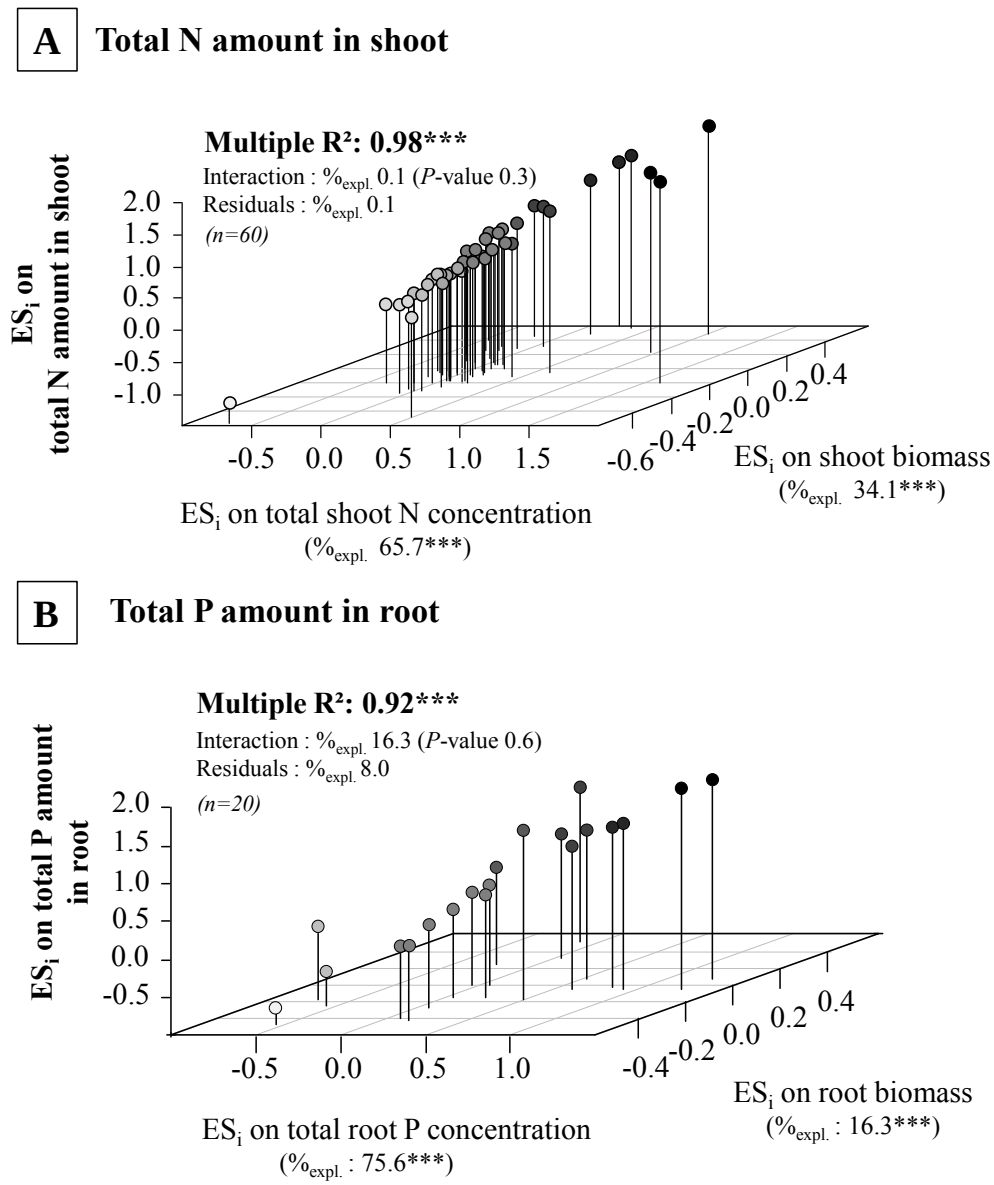


Figure 6. Changes in bacterivore-induced effect size (mean) on shoot (A) and root (B) biomass according to the presence of mycorrhizal fungi (-M : non mycorrhizal plant; +M: mycorrhizal plant). Different letters (a and b) indicate significant changes according to a Kruskal-Wallis test (P<0.05, n=14).

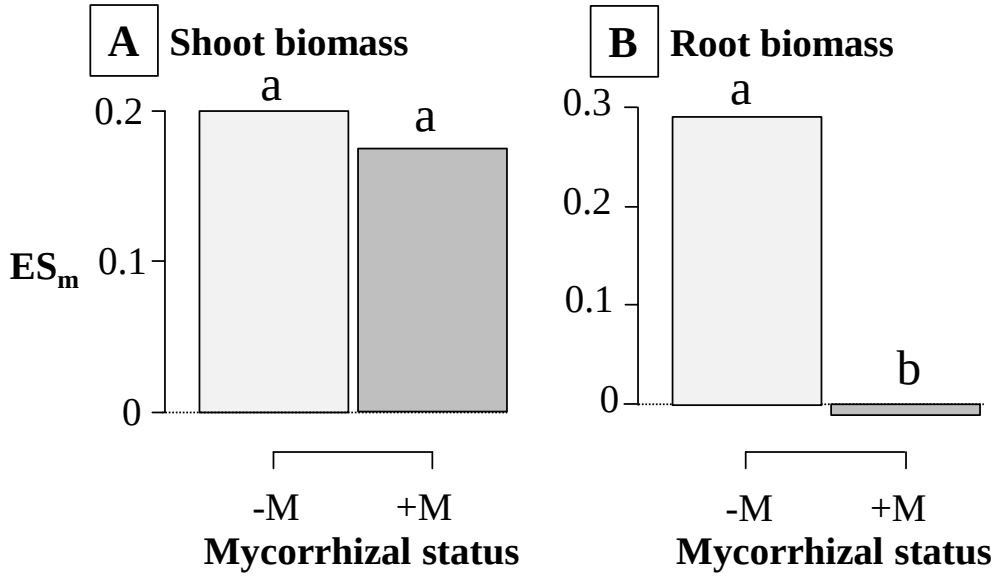
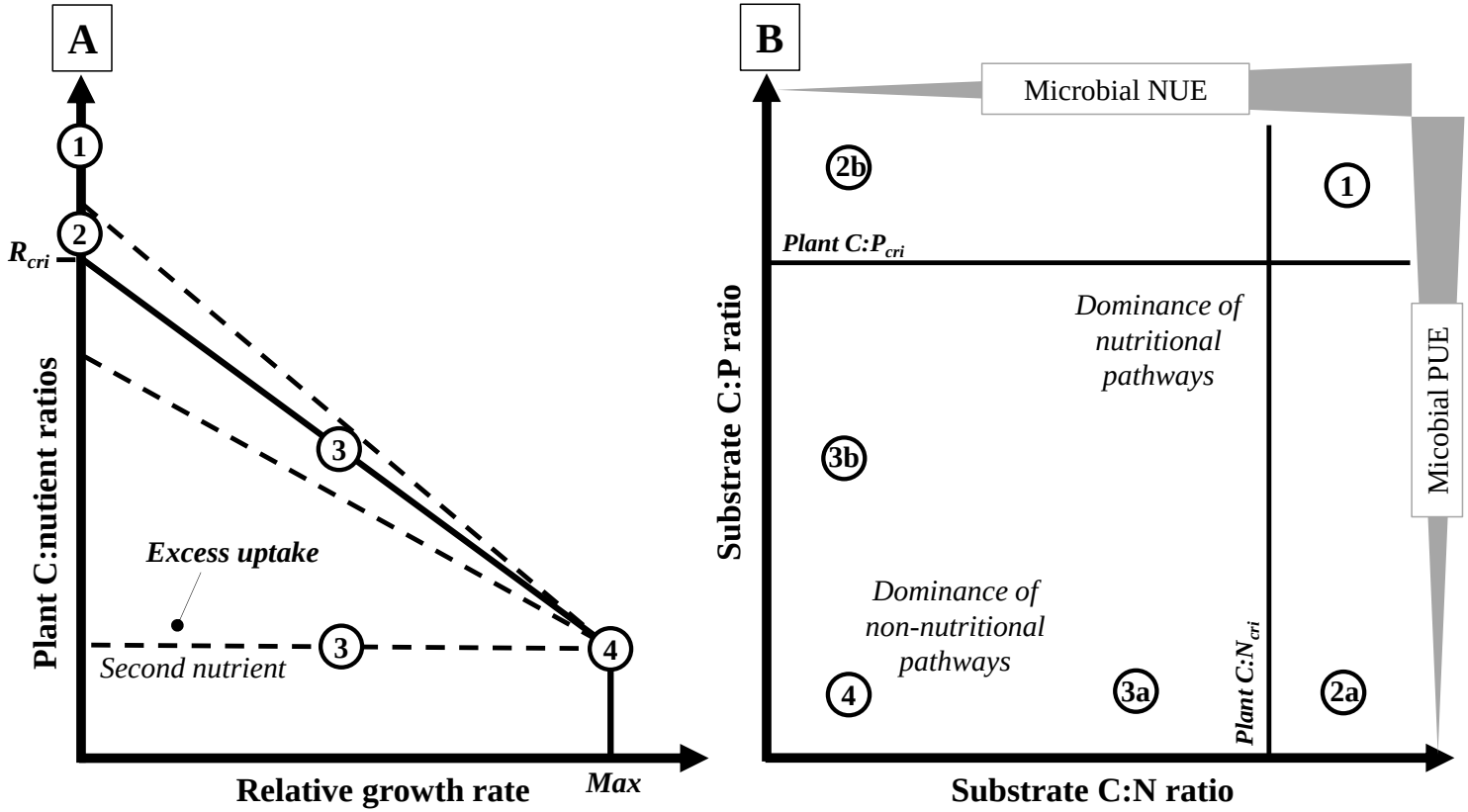


Figure 7. Theoretical framework describing the relative dominance* of nutritional and non-nutritional mechanisms involved in bacterivore-effects on plant performance.

(A) Relationship between plant relative growth rate and plant C:nutrient ratios (modified from Ågren (2008)). The solid line describes the C:limiting-nutrient (N or P) ratio while the broken lines indicate three different variations in the C:nutrient ratio of a second element (N or P) as a function of relative growth rate. We here defined the ***critical*** C:limiting-nutrient ratio (R_{cri}) above which the relative growth rate is null.

(B) Diagram illustrating four different cases of plant behavior in the presence of bacterivores along a soil C:N and C:P gradients. Solid lines describe the $C:N_{cri}$ and $C:P_{cri}$ according to soil C:N:P ratio, while the broken line indicates a hypothetical zone where the dominant mechanism changes. Grey arrows describe the hypothetical trends in microbial nitrogen- and phosphorus-use efficiency (NUE, PUE) along the soil C:N:P gradient. *the term “dominance” describes the relative importance of mechanisms to initiate and drive the effects of bacterivores on plants at a given moment in the experiment.



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Appendix 1. List of studies (in chronological order) used in the meta-analysis.

References	Year	Country	Medium	Bacterivores		Nutrients		Plant (species)	Mycorrhiza	Journals
				Protist	Nematode	Nitrogen	Phosphorus			
Coleman et al.	1977	USA	Soil	Yes	Yes	Yes	Yes	No	No	*Ecological Bulletins*
Cole et al.	1978	USA	Soil	Yes	Yes	No	Yes	No	No	*Microbial Ecology*
Elliott et al.	1979	USA	Soil	Yes	No	Yes	Yes	No	No	*Intern. J. Environmental Studies*
Cole et al.	1978	USA	Soil	Yes	Yes	No	Yes	No	No	*Microbial Ecology*
Anderson et al.	1981	USA	Soil	No	Yes	Yes	Yes	No	No	*Microbial Ecology*
Baath et al.	1981	Sweden	Humus	No	Yes	Yes	No	*Pinus sylvestris*	No	*Oïkos*
Woods et al.	1982	USA	Soil	Yes	Yes	Yes	No	No	No	*Soil Biology & Biochemistry*
Trofymow et al.	1983	USA	Soil	No	Yes	Yes	No	No	No	*Oecologia*
Anderson et al.	1983	USA	Soil	No	Yes	Yes	No	No	No	*Oïkos*
Clarholm	1985	Sweden	Soil	Yes	No	Yes	No	*Triticum sp.*	No	*Soil Biology & Biochemistry*
Ingham et al.	1985	USA	Soil	No	Yes	Yes	Yes	*Bouteloua gracilis*	No	*Ecological Monographs*
Griffiths	1986	Scotland	Soil	Yes	Yes	Yes	Yes	No	No	*Soil Biology & Biochemistry*
Kuikman & Van Veen	1989	Netherlands	Soil	Yes	No	Yes	No	*Triticum aestivum*	No	*Biology and Fertility of Soils*
Kuikman et al.	1990	Netherlands	Soil	Yes	No	Yes	No	*Triticum aestivum*	No	*Biology and Fertility of Soils*
Setälä et al.	1991	Finland	Humus	No	Yes	Yes	Yes	No	No	*Pedobiologia*
Kuikman et al.	1991	Netherlands	Soil	Yes	No	Yes	No	*Triticum aestivum*	No	*Soil Biology & Biochemistry*
Rutherford et al.	1992	Canada	Soil	Yes	No	Yes	No	No	No	*Canadian Journal of Soil Science*
Bouwman et al.	1994	Netherlands	Soil	No	Yes	Yes	No	No	No	*Biology and Fertility of Soils*
Jentschke et al.	1995	Germany	Sand + nutrient solution	Yes	No	Yes	Yes	*Picea abies*	Yes	*Biology and Fertility of Soils*
Alphei et al.	1996	Germany	Soil	Yes	Yes	Yes	Yes	*Hordelymus europaeus*	No	*Oecologia*
Ferris et al.	1998	USA	Sand-OM substrate	No	Yes	Yes	No	No	No	*Plant and Soil*
Setälä et al.	1999	Finland	Sand-OM substrate	No	Yes	Yes	No	*Pinus sylvestris*	Yes	*Oïkos*
Bardgett & Chan	1999	UK	Soil	No	Yes	Yes	Yes	*Nardus stricta*	No	*Soil Biology & Biochemistry*
Bonkowski et al.	2000	UK	Soil-sand-OM substrate	Yes	Yes	Yes	No	*Lolium perenne*	No	*Applied Soil Ecology*
Bonkowski et al.	2001a	UK	Soil-sand-OM substrate	Yes	No	Yes	Yes	*Triticum aestivum*	No	*Oïkos*
Bonkowski et al.	2001b	Germany	Humus	Yes	No	Yes	Yes	*Picea abies*	Yes	*Applied Soil Ecology*
Djigal et al.	2004a	Senegal	Soil	No	Yes	Yes	Yes	*Zea mays*	No	*Plant & Soil*
Djigal et al.	2004b	Senegal	Soil	No	Yes	Yes	Yes	*Zea mays*	No	*Soil Biology & Biochemistry*
Postma-Blaauw et al.	2005	Netherlands	Soil-OM mixture	No	Yes	Yes	No	No	No	*Oecologia*
Herdler et al.	2008	Germany	Soil	Yes	No	Yes	Yes	*Oryza sativa*	Yes	*Soil Biology & Biochemistry*
Somasundaram et al.	2008	Japan	Soil	Yes	No	No	No	*Oryza sativa*	No	*Plant Production Science*
Ekelund et al.	2009	Netherlands	Soil-OM mixture	Yes	No	Yes	No	*Holcus lanatus*	No	*Soil Biology & Biochemistry*
Krome et al.	2009a	Germany	Sand-OM mixture	Yes	No	Yes	No	*Arabidopsis thaliana*	No	*Soil Biology & Biochemistry*
Krome et al.	2009b	Germany	Agar/Sand	Yes	No	Yes	No	*Lepidium sativum & Arabidopsis thaliana*	No	*Plant & Soil*
Xiao et al.	2010	China	Soil-OM mixture	No	Yes	Yes	No	No	No	*Applied Soil Ecology*
Cheng et al.	2011	China	Soil	No	Yes	Yes	No	*Oryza sativa*	No	*Nematology*
Irshad et al.	2011	France	Agar	No	Yes	Yes	Yes	*Pinus pinaster*	No	*Soil Biology & Biochemistry*
Bjornlund et al.	2012	Denmark	Soil-OM mixture	No	Yes	Yes	No	*Hordeum vulgare*	No	*European Journal of Soil Biology*
Irshad et al.	2012	France	Agar	No	Yes	No	Yes	*Pinus pinaster*	Yes	*Plant and Soil*
Koller et al.	2013a	Germany	Soil-sand mixture	Yes	No	Yes	No	*Plantago lanceolata*	Yes	*Soil Biology & Biochemistry*
Koller et al.	2013b	Germany	Soil-sand mixture	Yes	No	Yes	No	*Plantago lanceolata*	No	*FEMS Microbiology Ecology*
Ranoarisoa et al.	2015	France	Soil-sand mixture	No	Yes	Yes	Yes	*Pinus pinaster*	Yes	*Personnal communication*